

# NMR and Molecular Mechanics of Alachlor Conformation and Conformers: Implications to the Mechanism of Environmental Photodegradation

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Hindered intramolecular free rotation around specific single bonds within alachlor accounts for the existence of the stable conformers observed in solution by NMR spectroscopy. NMR and molecular mechanics techniques enable identification of the molecular chemical environment under which specific conformations of alachlor are changed. Results suggest the photodegradation products previously reported have one of these conformers as a common intermediate. Chemical environments that increase the amount of this conformer could therefore enhance its rate of photodegradation. Chemical environments that stabilize a different conformation may have significantly longer half-lives or different photodegradation products.

**Keywords:** NMR; molecular mechanics; photodegradation; pesticide; alachlor

## INTRODUCTION

The herbicide alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide] has been found in ground and well waters (Chester et al., 1989; Bushway et al., 1992). In addition, aerobic and anaerobic conditions affect rates of its microbial degradation (Pothuluri et al., 1990), but rates of loss can also occur by physical adsorption and/or desorption from soils (Clay and Koskinen, 1990). Products of its metabolism have been detected in mammals (Jacobsen et al., 1991) and insects (Wei and Vossbrinck, 1992). Conformational changes on binding of other structurally labile compounds observed by NMR spectroscopy have been used as receptor site probes (Schmidt et al., 1993a). A common intermediate species has been postulated to explain the observed photochemical degradation products of alachlor (Somich et al., 1988).

Although alachlor contains primarily single bonds (but also a carbonyl and an aromatic ring), steric hindrance could limit free rotation among different conformers in solution. The chemical basis for its adsorption and the selection among different degradation pathways may be explained by stabilization of specific conformations at the molecular level. In this study the solution rotamers and conformers of alachlor were investigated by NMR spectroscopy and molecular mechanics to determine the conformational requirements that may be essential for photodegradation to occur. Identifying these requirements within a structurally simpler chemical environment could be indicative of the factors, rates, and mechanisms of its interaction within structurally more complicated environmental matrices.

## MATERIALS AND METHODS

**Chemicals.** Alachlor was provided by Monsanto Co., St. Louis, MO, and its purity was >99% by gas chromatography. Perdeuterated solvents of acetonitrile (ACN-*d*<sub>3</sub>), acetone (ATONE-*d*<sub>6</sub>), benzene (Bz-*d*<sub>6</sub>), chloroform (CDCl<sub>3</sub>), deuterium oxide (D<sub>2</sub>O), 1,2-dimethylbenzene (*o*-XYL-*d*<sub>10</sub>), 1,4-dimethylbenzene (*p*-XYL-*d*<sub>10</sub>), dimethyl sulfoxide (DMSO-*d*<sub>6</sub>), methanol (MeOD-*d*<sub>4</sub>), and toluene (TOLU-*d*<sub>8</sub>), were purchased from Isotec, Inc., Miamisburg, OH (each 99+% D). Remaining

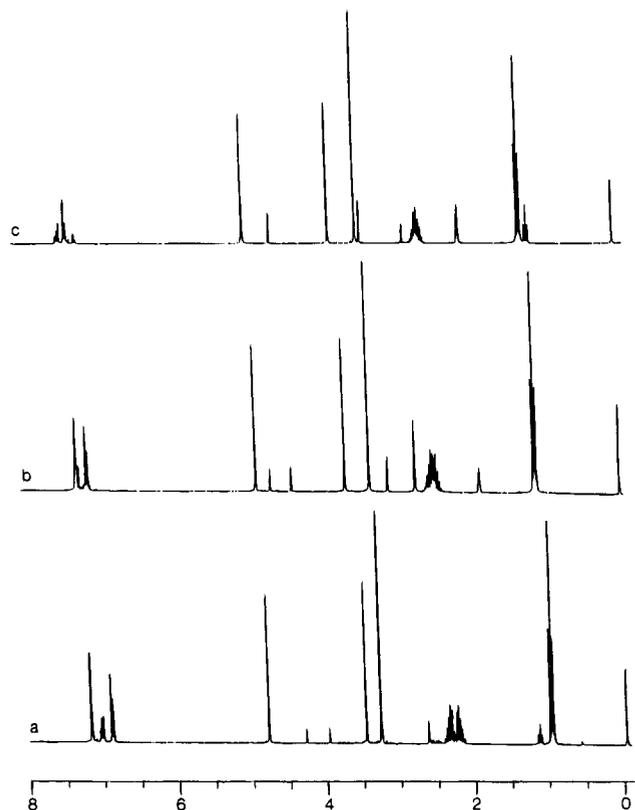
solvents were of spectrophotometric quality and purchased from Burdick and Jackson, Muskegon, MI.

**NMR Spectroscopy.** Solution NMR spectra were recorded with a Bruker QE Plus spectrometer (300 MHz for <sup>1</sup>H and 75 MHz for <sup>13</sup>C) with 32 acquisitions at a proton spectral width of 2500 Hz and at a carbon spectral width of 9600 Hz. The intramolecular assignment of chemical shifts for conformers was confirmed by 2D homonuclear correlation spectroscopy (COSY) and 2D heteronuclear correlation spectroscopy (HETCOR) (Derome, 1989). One-dimensional nuclear Overhauser enhancement (NOE) difference spectra (NOEDS) experiments and attached proton test (APT) (Nakanishi, 1990) were also conducted. All chemical shifts are referenced directly to internal TMS (0.0 ppm). Temperature of the samples was controlled to ±0.5 °C of set temperature.

Solutions of 8 mg/mL of alachlor were made in ACN-*d*<sub>3</sub>, Bz-*d*<sub>6</sub>, CDCl<sub>3</sub>, carbon tetrachloride, DMSO-*d*<sub>6</sub>, ATONE-*d*<sub>6</sub>, MeOD-*d*<sub>4</sub>, TOLU-*d*<sub>8</sub>, *o*-XYL-*d*<sub>10</sub>, and *p*-XYL-*d*<sub>10</sub>. The spectrometer locked onto the deuterium within the solvents. Binary solutions of solvents each containing an equal concentration of alachlor were used to influence alachlor conformations. The resulting changes in relative concentration among conformers were monitored using NMR spectroscopy.

**Molecular Mechanics.** The conformation of alachlor corresponding to global minimum energy was determined using molecular mechanics with Gasteiger–Marsili electrostatics in the Tripos force field in the computer program Sybyl 5.5 obtained from Tripos Associates, St. Louis, MO, on a Sun work station, Mountain View, CA. The computer calculates the steric energy within alachlor from an initial conformation and then makes small adiabatic conformational changes in the molecule to lessen intramolecular stress. From the resulting new alachlor conformation, the same procedure is repeated. This stepwise process concludes when any further conformational change increases global steric energy. The global minimum energy must be calculated from diverse starting conformations to prevent the assignment of a local minimum as the global minimum. The preferred conformation of atoms about every labile bond in alachlor was investigated as an additional initial conformation. From the energy-minimized conformation, rotating any labile bond up to 90° on computer reminimization results again in the global minimum conformation. Higher energy conformers are similarly determined. The molecular mechanics calculations were assumed to be in a vapor phase at room temperature with no significant field effects from neighboring molecules.

**Photodegradation.** Irradiations of 100 ppm of alachlor were in two solvents (ACN and Bz) and in binary mixtures of



**Figure 1.** Proton spectra of alachlor conformers: a, in Bz- $d_6$ ; b, in an equivolume mixture of DMSO- $d_6$  and Bz- $d_6$ ; and c, in DMSO- $d_6$ .

the solvents (50:50, 55:45, 70:30, 85:15, v/v) within an Ace Glass photochemical reactor using a Conrad-Hanovia medium pressure mercury arc lamp contained within a water-cooled quartz immersion well (Ace Glass Co., Vineland, NJ). A Pyrex filter sleeve was used to eliminate wavelengths below 290 nm. Quartz glass tubes were filled with 7 mL aliquots of the treatment solutions. Teflon-lined caps were used. At selected times after the start of irradiation, tubes were removed from the reactor. An aliquot was transferred to a sample vial for automated injection on a Hewlett-Packard 5890 gas chromatograph with flame ionization detection. The sample injection port was operated in the splitless mode at 220 °C, and 1  $\mu$ L sample volumes were injected onto a DB-1 capillary column (30 m  $\times$  0.32 mm i.d., 0.25  $\mu$ m film thickness). The oven temperature was programmed from 120 to 220 °C at a rate of 10 °C/min. Alachlor was quantitated by the external calibration method from three standards prepared with each of the six solvent conditions.

## RESULTS AND DISCUSSION

### NMR Spectroscopy and Molecular Mechanics.

Two distinct stable rotamer states of alachlor were observed in each of the individual solvents. Aliphatic frequencies for structurally equivalent protons were split into two corresponding frequencies of unequal intensity (Figure 1). Assignments of conformers to chemical structure among proton peaks (Table 1) are unambiguously confirmed by HETCOR and COSY. Each set of chemically equivalent protons between conformers was linked to a single corresponding carbon frequency because chemical exchange occurs between the different conformers. APT differentiates between methyl and methylene singles.

The two major observed rotamers are consistent with slow two-site chemical exchange; i.e. the Ar-N-C=O torsion angle flips between 180° (Figure 2a) and 0° (Figure 2b). The observation that within structurally

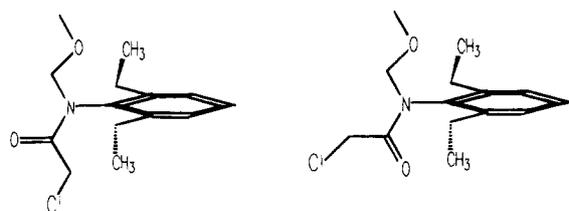
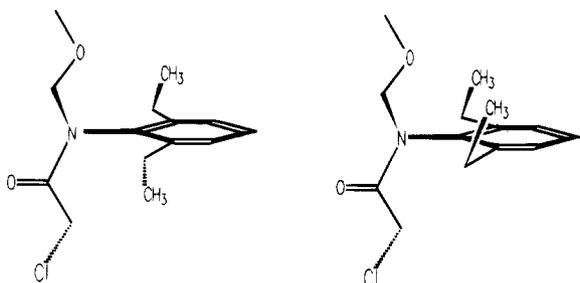
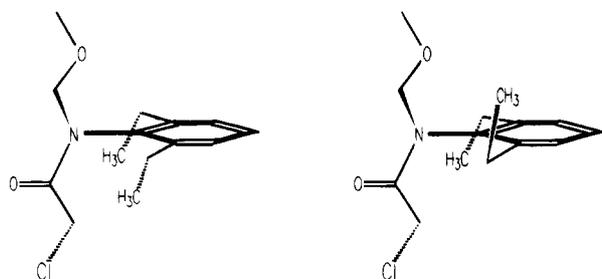
simpler anilides, the preferred conformation of the carbonyl is in a trans position to the aromatic ring (as in Figure 2a) has previously been reported (Brown et al., 1968). Either one or two chemical shifts for the protons with identical chemical structure could occur. Two rotamers would be experimentally indistinguishable in the NMR spectrum if fast exchange between the two conformations occurred (Harris, 1986). In its lowest energy conformation calculated by molecular mechanics, the aromatic ring is perpendicular to the aliphatic chain as shown in Figure 2a (3.9 vs 6.77 kcal/mol for Figure 2b). Steric hindrance increases strongly whenever the Ar-N-C=O torsion angle deviates from either 180° or 0°, which limits free rotation about the amide bond and about the Ar-N bond. Any double-bond character in the C-N amide bond would correspondingly inhibit free rotation. The large mass from the terminal chlorine atom should also slow the rate at which the carbonyl flips.

Intramolecular steric hindrance is an essential element for the existence of two conformationally distinct rotamers. The melting point of alachlor is 40–41 °C (Windholz, 1983). Temperature studies from 27 to 67 °C in DMSO- $d_6$  using NMR indicated that the relative populations of the two alachlor rotamers remain fairly constant despite the temperature change, and both are stable in solution at environmentally relevant temperatures. Changing the chemical environment by altering the solvent (e.g. from nonpolar aprotic CCl<sub>4</sub> to polar protic MeOH- $d_3$ ) also had no major effect on the relative populations of the Figure 2a,b conformers. Because in both DMSO- $d_6$  and ATONE- $d_6$  similar spectra were observed, viscosity was not a significant factor that altered these populations.

**Rotamer and Conformer Structure.** The two spectral features unexplained by the carbonyl conformers are the aromatic ring and the two ethyl groups attached to the ring. Three aromatic proton frequencies are discernible in DMSO- $d_6$  (Figure 1c). Symmetrical conformations and/or dynamics would predict that both aromatic protons in an ortho position relative to the substituents should be indistinguishable. The terminal CH<sub>3</sub> on the two ethyl groups (which should be a triplet) bound to the aromatic ring in alachlor becomes two triplets of unequal intensity, consistent with slow exchange between two conformers. Molecular mechanics, which uses only vapor phase molecular parameters, demonstrated that the CH<sub>3</sub> is most stable when perpendicular to the aromatic ring (Figure 3). The maximum steric hindrance occurs with the CH<sub>3</sub> in the same plane as the aromatic ring. Above and/or below the plane of the ring, the CH<sub>3</sub> protons have minimum steric effects on the hindrance of the mobility of the aliphatic chain. Four stable conformers exist for the positions of the CH<sub>3</sub> groups. Two forms (Figure 3a,d) are symmetrical (identical mirror images and identical in steric energy) for both the aromatic and methyl protons and are not distinguishable in the NMR experiment. The remaining two conformers are asymmetrical (nonsuperimposable mirror images) for both the aromatic and methyl protons (Figure 3b,c) with Figure 3b lower in energy. Using a Boltzmann distribution to predict thermal energy (Harris, 1986), a population difference between two conformer states of 1/10 corresponds to a difference in thermal energy of 1.38 kcal/mol. These results are of the same magnitude as those calculated by molecular mechanics in the vapor phase. Thus,

**Table 1. Proton Chemical Shift Assignments of Alachlor Conformers**

chemical structure	spectra in DMSO- <i>d</i> <sub>6</sub> (Figure 1c)		spectra in 1/1 mixture (Figure 1b)		spectra in benzene- <i>d</i> <sub>6</sub> (Figure 1a)	
	multiplicity	$\delta$ (ppm)	multiplicity	$\delta$ (ppm)	multiplicity	$\delta$ (ppm)
CH <sub>2</sub> CH <sub>3</sub>	triplet	1.16	triplet	1.13	triplet	0.98
	triplet	1.25	triplet	1.13	triplet	1.17
CH <sub>2</sub> CH <sub>3</sub>	multiplet	2.50	multiplet	2.48	multiplet	2.21
	multiplet	2.50	multiplet	2.48	multiplet	2.32
OCH <sub>2</sub> N	singlet	3.26	singlet	3.12	singlet	2.65
	singlet	3.42	singlet	3.32	singlet	3.27
CH <sub>2</sub> Cl	singlet	3.81	singlet	3.77	singlet	3.47
	singlet	4.66	singlet	4.64	singlet	3.97
OCH <sub>3</sub>	singlet	4.81	singlet	4.72	singlet	4.28
	singlet	4.84	singlet	4.86	singlet	4.78
Ar-H	multiplet	7.17	multiplet	7.12	multiplet	6.89
	multiplet	7.26	multiplet	7.29	multiplet	7.04
	multiplet	7.36				
residual solv	singlet	2.54	singlet	2.54	singlet	7.23
				7.23		

**a: Steric Energy 3.95 kcal/mol****b: Steric Energy 6.77 kcal/mol****Figure 2.** Two lowest energy stable conformations of alachlor from 180° carbonyl rotation calculated by molecular mechanics. All intermediate states of the carbonyl rotation are higher energy conformers.**a: Steric Energy 3.95 kcal/mol****b: Steric Energy 4.28 kcal/mol****c: Steric Energy 5.43 kcal/mol****d: Steric Energy 3.95 kcal/mol****Figure 3.** Four lowest energy stable conformations of alachlor from 180° rotation of the terminal CH<sub>3</sub> on the ethyl ring substituents. Every intermediate state of the ethyl ring substituent rotation is a higher energy conformer.

solvent effects cannot be considered the primary cause of the separate alachlor conformers.

The carbonyl rotamers and the methyl conformers could in principle change independently. An alternative

proposal would be that methyl conformers are directly related to the higher energy carbonyl rotamer (i.e. Figure 2b). Experimental evidence supports the first premise. The smaller methyl peak was to the right (shielded) relative to the second methyl frequency in Bz-*d*<sub>6</sub>, whereas in DMSO-*d*<sub>6</sub> the smaller was to the left (deshielded) relative to the more abundant frequency. In binary solution of the solvents (each containing identical amounts of alachlor), only one of the conformers was observed (Figure 1b). The three aromatic protons coalesced into two frequencies. The methyl conformers and aromatic frequencies could be titrated in binary solutions of an anisotropic aromatic solvent (benzene, toluene, *o*-xylene, or *p*-xylene) with nonaromatic solvents (acetone, acetonitrile, dimethyl sulfoxide, methanol, chloroform, or carbon tetrachloride). Diverse chemical environments can alter the methyl conformer without appreciably altering the carbonyl rotamer conformation.

Second, NOE measurements between the methyl conformers and the alpha aromatic proton confirm that state 1b is not the arithmetic average of states 1a and 1c. NOEDS experiments are used to examine closeness in space among sites within a molecule (Nakanishi, 1990). When only one methyl conformer is apparent (i.e. in the binary 50:50 solvent mixture), the NOE on the aromatic region was lowest (4%). When the solvent was changed stepwise to only Bz-*d*<sub>6</sub>, the NOE to the aromatic region for each conformer was doubled (8%). In pure ACN-*d*<sub>3</sub>, an even larger NOE (12%) was observed and the first two aromatic peaks at 7.17 and 7.27 ppm are spatially linked to a methyl group. An NOE was observed with both methyl triplets to both methines, indicating rotational exchange also occurs during the experiment between the two conformers. In solvent mixtures between the 50:50 Bz-*d*<sub>6</sub>/ACN-*d*<sub>3</sub> and pure ACN-*d*<sub>3</sub>, NOE intermediate enhancements occurred (between 4% and 12%). A chemical environment that makes alachlor conformers more symmetrical decreases the contact between the methyl groups and the aromatic ring. These results indicate that the methyl groups flip less often when only one conformer is observed. Both pure aromatic and pure nonaromatic solvents stabilize conformations, each of which are asymmetrical, but these two asymmetrical conformations are not identical.

**Solvent Structure.** In both carbon tetrachloride and deuterioacetonitrile, the same asymmetrical conformer



states (3c or 3b) were equally favored by a localized intra- or intermolecular chemical environment.

From the double bond character of the amide bond, partial conjugation between the  $\pi$ -bonds of the carbonyl group and the  $\pi$ -bonds of the aromatic ring occurs. Since carbonyl  $\pi$ -bonds and the aromatic ring  $\pi$ -bonds both have a positive and a negative component, under slow rotational exchange among the four conformations, the net localized anisotropy does not necessarily average to zero.

Unequal dynamics in the methyl ring flips could be a cause or a result of such anisotropy. From structure 3a, the front methyl group may flip more readily than the back one. From structure 3d, the back methyl group may flip more readily than the front one. Unequal ring side chain mobility would induce a small torque and temporarily alter the 90° angle between the aliphatic chain and the ring. This in turn would allow more space for the same methyl group to flip and concurrently less space for the opposite methyl group. At an angle of 95°, one methyl group would have greater mobility; at 85° the mobility of the opposite methyl group would be favored. The angles of 95° and 85° are neither structurally nor mathematically equivalent in an anisotropic environment.

**Photodegradation.** The loss of alachlor upon UV irradiation was quantitated from combinations of acetonitrile and benzene (Table 2). An appreciable half-life was measured (900 h) with 30% benzene. At 45% benzene and above, no photodegradation was observed over the same period. In contrast, increasing the amount of acetonitrile from 85% to 100% decreased the half-life step wise by factors of 4 (220 h) and 5 (43 h), respectively. Thus, benzene significantly lowers the rate of photodegradation.

One explanation is that benzene could competitively absorb UV light, inhibiting alachlor photodegradation. However, NMR data would suggest that effective competitive absorption is not the only possible mechanism. The conformation stabilized within the two chemical environments could determine the photodegradation mechanism because the conformers in aromatic solvents are different from those in nonaromatic solvents. In 30% benzene with and without alachlor, the UV absorbance from benzene at 290 nm was less than 0.050 AUFS. At 30% benzene (acetonitrile/benzene/alachlor: :2300/1000/1 mole ratio), significant photodegradation still occurred.

Different conformers could be significantly different in their sensitivity to photodegradation. We propose that a specific asymmetrical conformation of alachlor could be a critical requirement for the photodecomposition to occur. In conformation 3b with both methyl groups above the ring, space for the carbonyl rotation increases. Concomitantly, one  $\text{CH}_2\text{CH}_3$  hydrogen ( $\text{H}_a$ ) (Scheme 1) is spatially close to the carbonyl or the  $\text{CH}_2\text{-Cl}$  hydrogen. A 10–15° tilt between aliphatic chain and aromatic ring from 90° brings them to distances at which their localized electronic fields could interact. The second "up" methyl group is simultaneously physically close to the methoxy oxygen. Because of the carbonyl amide type bond, not just the  $\text{C=O}$  but the entire  $\text{C-N-(C=O)-C}$  is planar. The methyl groups distorting this planarity may inherently weaken the  $\text{(C=O)-C-Cl}$  bond which is the initial site of the photodegradation. Upon abstraction of the  $\text{H}^\bullet$  from the same conformation, ring closure would result in the previously isolated photodegradation product 3. With

carbonyl rotation (and addition from water of  $\text{H}^\bullet$  or  $\text{OH}^\bullet$ ), other products 1 and 2 would result. The resulting compounds in Scheme 1 are the same products previously proposed to explain the photodegradation products of alachlor (Somich et al., 1988).

In contrast, structure 3c, in which both of the methyl groups are down, would not give rise to the same products. Steric effects would prevent tilting of the aliphatic chain more than several degrees, and because the  $\text{CH}_2\text{Cl}$  group is closer to the  $\text{CH}_2\text{CH}_3$  than the  $\text{CH}_2\text{-CH}_3$  hydrogens, the conformation would be stabilized rather than destabilized. Symmetrical conformers such as 3a have half the correct methyl conformations. The isomer, however, cannot be physically or experimentally distinguished from its mirror image (structure 3d) unless the environments of the two states are chemically different. This could occur in a chiral environment (Schmidt et al., 1993b, 1994). However, it could also be that the symmetrical conformers are the most stable because they are symmetrical. Thus, the alachlor half-life in chemical environments that favor the asymmetrical conformer 3b could be much shorter than those which favor conformer 3c or the symmetrical conformations.

**Conclusions.** Hindered intramolecular motion of alachlor results in several stable conformers present in solution and observable by NMR spectroscopy. Changes in conformation can be induced by altering the solution chemical environment. NMR and molecular mechanics enable identification of the molecular conditions and chemical environment under which specific alachlor conformers are stabilized. Environmental factors that affect the conformation of alachlor at the molecular level may alter both the rate and pathway of alachlor degradation. Both the rates and degradation pathways (and products) may be affected by which conformations are most stabilized. Such information could prove to be useful in identifying components in more complex chemical environments that hinder or enhance alachlor half-life.

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